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# Visual perception of texture in aggressive behavior of Betta splendens( Dissertation\_全文 )

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CITATION:

Bando, Toshihiro. Visual perception of texture in aggressive behavior of Betta splendens.  
京都大学, 1991, 理学博士

ISSUE DATE:

1991-05-23

URL:

<https://doi.org/10.11501/3054081>

RIGHT:

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Visual perception of texture in aggressive behavior of Betta splendens.

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Running title: Texture perception in fish.

Summary. In order to elucidate the role of the textures in fish vision, scale pattern recognition of fish was examined using the visual model made by image processing technique. Male Siamese fighting fish (Betta splendens) were used as subjects because they exhibit clear agonistic behaviors to the sight of conspecific males.

In the study of reaction to the side view model (the images with contour shape of B. splendens' side view in aggressive stage), B. splendens responded less vigorously to the space averaged images in which we can only see global brightness and color distribution, but cannot see relatively fine features like scale pattern.

Aggressive behaviors of the fish were also measured by the circular model which have circular contour shape with and without scale pattern mapping and spherical gradation on them. The images with the scale pattern evoked more aggressive behaviors than those without it, while the existence of spherical gradation affected the behavior slightly.

These results suggest that texture plays an important role in fish visual perception.

Key words: Betta splendens - Aggressive behavior - Scale pattern - Texture - Visual recognition.



## Introduction

Numerous studies on fish vision has been accumulated in the last thirty years. Those investigations include color recognition (Hawryshyn et al. 1988; Loew and Lythgoe 1978; Silver 1974; Tomita et al. 1967; MacNichol 1964), orientation discrimination (Mackintosh and Sutherland 1963; Volkman and Zametkin 1974), recognition of partial pattern like eye-spot (Altbäcker and Csányi 1990; Coss 1979; Kohda and Watanabe 1990) and contour shape discrimination (Sutherland 1969). Stimuli used in those experiments were mainly composed of simple and monotonous color planes or straight lines. Though these studies give us basic information to understand the mechanism of fish vision, the animals seldom encounter such simple patterns in their natural environment.

In contrast to the artificial environment constructed with simple geometrical shapes and monotonous planes, natural environment includes many objects constructed with mosaic of various brightnesses and colors. It would be reasonable to assume that there are many visual cues in natural environment that animals can utilize for their survival. In this context, it is important to investigate animals' ability to discriminate features of mosaic patterns made of various brightnesses and colors (i.e. textures). Since it is difficult to define or analyze such natural patterns quantitatively or to make suitable experimental stimulus patterns, there has been very few detailed studies on animals' recognition of natural patterns.

Due to the progress in image processing technique, there are some reports on man or animal vision in which texture patterns were used as stimuli. But in most of them, texture has been used to make random dot stereogram (Julesz 1964), as an element of edge or plane (Tooell et al. 1988; Frost et al. 1988), not to examine the role of the texture itself in visual perception of natural environment.

In this experiment, focusing on the scale pattern on the trunk of the fish, I investigated the importance of texture in visual perception of the fish. I chose the scale pattern because i) this pattern is the main texture on the trunk, ii) the repetition of dark and light color made almost uniform pattern on the trunk, and iii) this pattern is stabler than the striate patterns on the fins which change time to time with the movement of fins. Male B. splendens was used as experimental animal because it exhibits a very clear aggressive behavior to the conspecific male, induced mainly by the visual cues (Simpson 1968). Photographs of the original and modified body patterns of the fish were prepared using image processing technique, and the behavior of the fish to those quantitatively defined patterns was analyzed to see if the textures which the fish actually see play an important role in their visual perception.



## Materials and Methods

Subjects. Four adult domesticated male B. splendens were obtained from a local supplier. They measured about 4-4.5 cm from snout to the base of caudal fin and their bodies were all blue. Fish were maintained individually in small tanks containing about 800 ml of aged tap water and were visually isolated from other animals. Water temperature was maintained at  $27 \pm 2^{\circ}\text{C}$ , and fish were fed daily with dried blood worm.

Apparatus. The test tank, 10x10x10 cm in size, was made of opaque glass except for its one side which was made of transparent glass. The stimulus pattern holder was kept 1 cm apart from the transparent side of the test tank. Opaque plastic shutter board was sustained between test tank and stimulus holder, and was pulled up and down by a pulse motor controlled by a microcomputer. To avoid the reflection of the image of the fish itself at the border of glass and air, the test tank and the stimulus pattern holder were put in a larger tank filled with water. Reactions of the fish were monitored and recorded by a video camera kept above the test tank. The test tank was illuminated by overhead fluorescent lamps (high color rendering index type, Toshiba FL40-S W-EDL-50K) and illumination at the surface of the test tank was about 600 lx. All the apparatus, except for control and monitoring parts, were set in a small darkroom to keep a constant light condition and to avoid the effect of experimenter.



Stimulus patterns. Two types of models were made for visual stimulus patterns: side view model and circular model (Fig.1). The side view model was made from a photograph of a side view of blue male B. splendens in aggressive stage. The image of the color photograph was transferred to the frame memory of the image processor through the color video camera. This image with white background was used as the original image of all side view patterns (S-O). All the side view patterns had the same contour shape as S-O, and filled with the 15x15 space averaged pattern (S-F15), the 45x45 space averaged pattern (S-F45), the average brightness and color (S-A), and the scale pattern (S-S).

Here, "NxN space averaged" means that the brightness of a point is equal to the average brightness of the NxN rectangle points area around it. The average brightness and color was determined by averaging each red (R), green (G) and blue (B) intensity value of all the pixels of the Betta's original image. To make S-S, a block of the scale pattern was cut off from the center part of S-O and a larger scale pattern plane was produced by parallel movement of this block. Series A stimulus pattern set consisted of these five side view patterns.

All circular models had a circular contour shape, and their inside were mapped spherically with the scale pattern the brightness distribution of which was the same as that of S-S with spherical gradation (C-SG), filled only with the spherical gradation (C-G), filled with the average brightness and color (C-A), and mapped with the scale pattern without



spherical gradation (C-S). All these patterns had the same color (i.e. the same R:G:B ratio as the average R:G:B ratio of the original image (S-O)). These patterns were made on the frame memory of the image processor using computer graphic technique from the data of the original image (S-O). The spherical gradation was calculated in the condition that the light source was 45 degrees above and in front of the sphere. Series B stimulus pattern set consisted of these four circular patterns and one side view pattern S-O as a control.

Four black and white patterns SBW-O, SBW-F15, SBW-F45 and SBW-A were made by averaging the brightness of R, G and B for each pixel from S-O, S-F15, S-F45 and S-A respectively, and used as series C stimulus pattern set. Therefore the brightness distributions of SBW-O, SBW-F15, SBW-F45 and SBW-A are the same as those of S-O, S-F15, S-F45 and S-A respectively which are shown in Fig.1.

These thirteen images on the color monitor were photographed, and their life size color prints were used as visual stimulus patterns. All these patterns have the same area and the same mean brightness (i.e. the mean of all R, G and B components value of all pixels).

Procedure. Fish were transferred to the test tank at least thirty minutes before the tests to adapt to the environment. The stimulus patterns were set on the holder behind the shutter board, and each test was started by pulling up the shutter when the fish swam across the center line of the test tank toward the stimulus pattern.



Subjects were exposed to a stimulus pattern for 3 minutes and their behaviors were recorded by the VTR for later analysis. Inter stimulus intervals were more than 2 minutes and subsequently the next exposure was started when the fish swam across the center line.

Five patterns of each series were exposed to the subjects once in each session and the order of presentation of the patterns was determined by a randomized block design in order to minimize the possible effect of ordering. Three sessions were repeated for each series of stimulus pattern and each fish, and inter session intervals were more than 4 hours.

#### Reaction indices.

To measure the aggressive behavior of B. splendens, three indices were assayed from the replay of the videotapes: i) display index (frequency of frontal and lateral display cycle), ii) movement index, and iii) area index.

#### Display index.

Frequency of frontal and lateral display cycles in response to the stimulus in 3 minutes were measured as display index. B. splendens shows stereotyped aggressive display toward the sight of the conspecifics, and repetition of this facing-broadside cycles is clear and easy to count (Fig.2a).

#### Movement index.

In the aggressive stage usually the movement of the fish increases. To evaluate this movement quantitatively and objectively, image processing techniques were used. From the

replay of VTR, a pair of images 10 frames (i.e. 333 ms) apart were transmitted to the frame memory of the image processor (nexus 6400) every second, and they were then binarized to detect the silhouettes of the fish. Logical operation XOR (exclusive OR) of the two binary images, the result of which is equivalent to unoverlapped parts of two silhouettes (Fig. 2b), were made and the area of these operated images were measured by the image processor repeatedly for 180 pairs (3 minutes) of images.

#### Area index.

In the aggressive stage the fish usually spread their fin. In order to detect the fin expansion quantitatively and objectively, VTR images of the fish were transmitted to the frame memory of the image processor every second, and they were then binarized for detecting the silhouettes of the fish (Fig.2c). The area of these operated images were then measured by the image processor repeatedly for 3 minutes. The sum of these values was used as the area index.

#### Statistical analysis.

In each series, the difference of response to each stimulus pattern was measured for the three behavioral indices using Wilcoxon's signed rank test (Siegel 1956).



## Results

### Aggressive responses to the stimulus and three reaction indices.

The fish in front of the stimulus patterns exhibited various aggressive behaviors, which contained spreading of fin, gill cover erection, repetition of frontal and lateral display, bite, tail beat, etc.

Among these behaviors, repetition of frontal and lateral display was one of the most clear and stable responses. This stereotyped response seemed to be correlated with the total level of other components of the aggressive behaviors and was a good indicator of aggressive activities.

The fish in aggressive stage moved actively in front of the stimulus patterns. This movement mainly consisted of the repetition of frontal and lateral display and the tail beat. Movement index measured by image processing reflected well the movement of the fish and seemed to indicate the level of the aggressive behaviors as well as display index.

Fin expansion was another frequent and clear response to the stimulus that could be easily detected. Though the area index also reflected the difference of activities in aggressive behaviors, it was not so sensitive in the present study. One of the reasons why it was not so sensitive seems to be that in this case the top view images were used to measure the area of the fish, thus the vertical spreading of the fin could not be detected without the tilting of the fin accompanied by the movement.



### Responses to series A stimulus patterns.

The results of the experiment on series A stimulus patterns are shown in Fig.3 and Table 1 with the results of multiple comparison by Wilcoxon's signed rank test. Though all stimulus patterns were 2-dimensional and still, subject fish exhibited clear aggressive responses to these patterns.

All three indices show that the original pattern (S-O) induced significantly more responses than the other four processed stimulus patterns. Display index and movement index show that, though the 15x15 space averaged pattern (S-F15) induced more responses than the side view pattern with the scale pattern (S-S), the difference between them is not significant. These two indices also show that S-F15 and S-S induced significantly more responses than S-F45 and S-A, and S-F45 was more effective than S-A.

Area index was not significantly different in S-S, S-F45 and S-A.

### Responses to series B stimulus patterns.

Circular models also induced aggressive behaviors regardless of their circular contour shape and unnatural global distribution of brightness and color, though significantly less than those of S-O.

The behavioral difference to the two types of models is that the subject fish tend to come close to the head part of the side view model, while it does not do so in the case of circular model, because circular model is symmetrical.

The results of the experiment on series B stimulus patterns are shown in Fig.3 and Table 2. Within the circular



model, relatively strong responses were induced by the patterns with the scale pattern (i.e. C-SG and C-S). Display index and movement index show that C-SG and C-S induced significantly more responses than C-G and C-A. The models with gradation did not induce significantly more responses than the models without it (C-SG vs. C-S, and C-G vs. C-A).

Area index was not significantly different in C-S, C-G and C-A.

#### Responses to series C stimulus patterns.

The results of the experiment on series C stimulus patterns are shown in Fig.3 and Table 3. Though all stimulus patterns were black and white, subject fish exhibited clear aggressive responses to these patterns.

The original black and white pattern (SBW-O) induced strong aggressive behaviors comparable to that of colored original pattern (S-O). Although the intensity of behaviors to the processed ones, SBW-F15, SBW-F45 and SBW-A also decreased in this order as in the case of series A and statistically significant in display index, level of aggressive behavior was clearly less than those of series A.

All three indices show that the original pattern (SBW-O) induced significantly more responses than the other four processed stimulus patterns. The display index also shows the significance of the difference in the behaviors induced by SBW-F15, SBW-F45 and SBW-A, while movement index and area index do not show any significant differences among these three patterns.



## Discussion

### Role of distribution of brightness and color of the stimulus patterns.

The results of the response to the series A stimulus patterns show that there is statistically significant difference between the agonistic behavior induced by the original pattern (S-O) and the one induced by the pattern of average brightness and color (S-A), indicating that the distribution of brightness and color plays an important role in Betta's visual recognition. The notions "brightness" and "color" in this paper are used for the physical properties of the stimulus patterns.

According to the notion of "average brightness and color" in this experiment, there is a possibility that averaging makes different tones of color, as in the case of fish with almost the same area of yellow and blue body color the average body color becomes gray, and affects the recognition and response of the fish. In the present case, however, "average color" is not so different from the original blue, because blue is dominant in the original pattern and only some parts of fins are red. This kind of effect is not so serious in this case and the main difference between S-O and S-A would be the distribution of brightness and color.

The fact that the circular model with the scale pattern (C-SG and C-S) elicited significantly stronger response than the one with uniform brightness (C-A), confirmed the importance of brightness distribution, because all circular



models had the same color, average brightness, contour shape and area.

The fact that the original black and white pattern (SBW-O) induced strong aggressive behaviors and the black and white pattern of average brightness (SBW-A) induced very weak responses also suggests the importance of brightness distribution. Although SBW-O induced as strong aggressive behaviors as those induced by S-O, this does not necessarily mean that color has no meaning in inducing aggressive behaviors, because SBW-F15 and SBW-F45 induced fairly weak responses as compared to those induced by S-F15 and S-F45 respectively.

#### Role of the low frequency components.

Main difference between S-F45 and S-A is global distribution of brightnesses and colors. The difference in brightness distribution between them can be seen in Fig.4, where the horizontal brightness distribution along the center line of the bodies are shown. The result that S-F45 induced more responses than S-A did indicate that global distribution of brightness and colors is one of the features B. splendens can recognize. Results of SBW-F45 and SBW-A support this with respect to the distribution of brightnesses, since they have the same brightness distribution as S-F45 and S-A respectively.

#### Role of the high frequency components.

Statistically significant difference between agonistic



behavior induced by the original pattern (S-O) and that induced by the 15x15 space averaged pattern (S-F15) indicate that relatively fine brightness and color distribution plays an important role in Betta's visual recognition. The difference between these two patterns in horizontal brightness distribution also can be seen in Fig.4. Power spectrum of brightness distribution in Fig.5 indicates that spectrum peak at 0.17 (1/pixel) is the main difference between them, although smaller peaks are seen in S-O. In this case, this peak of spectrum is related to the repetition cycle of the scale pattern, which suggests the importance of the scale pattern in Betta's visual recognition. This suggestion is supported by the fact that side view model entirely filled with the scale pattern (S-S) induced relatively strong aggressive responses regardless of the lack of other features. The results of black and white patterns SBW-O and SBW-F15 also support this, because they have the same brightness distributions as those of S-O and S-F15 respectively.

In this experiment the subject fish in aggressive behavior were swimming around 1 to 4 cm from the stimulus pattern. The spectrum peak at 0.17 (1/pixel) corresponds to 0.17 - 0.70 cycle/degree in visual angle when the subject sees the stimulus patterns from the point 1 - 4 cm apart from them. Our unpublished histological data of retina indicate that the adult fish of this species has the minimal separable angle of 45', which corresponds to spatial frequency of 1.33 cycle/degree. These values suggest that the subject fish have the ability to detect this texture pattern in this situation.



### Role of the contour shape.

Johnson and Johnson (1973) suggested that contour shape alone does not have a differential effect on Betta's aggressive behavior, using silhouette pattern of side view of the fish. The fact that C-SG and C-S induced relatively strong aggressive behavior regardless of their circular contour shapes suggest that contour shape is not an essential feature for B. splendens to judge if it is the object to fight with or not.

Though we think that the side view is the typical shape of fish, they show a variety of shapes depending on their relative position or movement. It would be reasonable to assume that the fish need other visual cues such as the scale pattern to recognize the species.

### Importance of the texture patterns and the techniques of stimulus pattern generation.

Since Tinbergen (1948) showed that the red belly of male three-spined stickleback releases fighting behavior, functions of color pattern of the fish has been studied in many species. Maeda and Hidaka (1979) indicated, using simple solid models, that parr marks play an important role in releasing aggressive behavior of Japanese trout, Oncorhynchus masou. The importance of the color pattern on the body in visual perception of male B.splendens was suggested by Robertson and Sale (1975), where solid models with "aggressive", "submissive", and "reproductive" color patterns elicited different responses. In these studies, they paid attention to the simple and clear



patterns like parr marks or longitudinal or vertical bars to release aggressive behavior, and not to the fundamental features in the body like scale pattern. These results showed even such simple color patterns can act as a releaser of important behavior; however, that does not necessarily mean that fish can not recognize fine or complex features of the color patterns.

The results of this study indicate that texture pattern like scale pattern is one of the key features in visual perception of B. splendens. In other words, relatively complex features in the patterns in the natural environment play important roles in animal's visual perception. The importance of detailed pattern that constitutes body texture pattern in Betta's visual perception has already been suggested in the study of Lissmann (1932). However, limitation of technique in making stimulus pattern did not permit precise evaluation of it. He compared the reaction of the fish to a series of the drawings where the elemental patterns of body and fins (e.g. fin striation, scale-like pattern) were added one by one to the outline shape of the fish. He found that the reaction of the fish gradually increased as the reality increases with the additions of the elemental patterns including scale-like pattern. However, we can not specify the key features to affect the reaction definitely with these kind of stimulus patterns, because brightness of the pattern also changes as he adds one of those elemental pattern. Image processing and computer graphics techniques made it possible to deal with color, brightness,



contour shape and area etc. very precisely. In the present study, I applied these techniques to make some sets of stimulus patterns which were different only in one or two features, keeping other features unchanged. These stimulus patterns made precise evaluation of fish visual perception feasible.

Acknowledgments. I wish to thank Dr. N. Matsumoto of Osaka University for his comments on this manuscript, and Dr. H. Tamura of Kyoto Institute of Technology for his stimulating suggestion. This research was supported by a grant from the Ministry of Education, Science and Culture of Japan (No.59760143).

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## Figure captions.

Figure 1. Visual stimulus patterns. S-O: Side view model of the original pattern, S-F15: Side view model filled with 15x15 spatial averaged pattern, S-F45: Side view model filled with 45x45 space averaged pattern, S-A: Side view model filled with average brightness and color, S-S: Side view model filled with scale pattern (taken from the mid body part of S-O), C-SG: Circular model with scale pattern mapping and spherical gradation, C-G: Circular model with spherical gradation, C-A: Circular model filled with average color, C-S: Circular model with scale pattern mapping. The original pictures of series A and B used in the experiment are the color pictures in which blue is dominant. The patterns of black and white stimulus set (series C) SBW-O, SBW-F15, SBW-F45 and SBW-A have the same brightness distributions as those of S-O, S-F15, S-F45 and S-A respectively. Calibration: diameter of the circle, 44 mm.

Figure 2. Measures of B. splendens' aggressive behavior as scene from the top. a: frontal and lateral display cycle toward the pattern (P). b: Movement index. Outline of two images 10 frames (= 1/3 sec) apart (left) and the result of logical operation XOR of the two images (summation of unoverlapped parts; dark parts in the right) c: Examples of B. splendens' silhouettes before (3 left panels) and during (3 right panels) aggressive behavior.

Figure 3. Responses of the fish to three series of the stimulus patterns.

Figure 4. Horizontal brightness distributions of visual stimulus patterns. In this figure "brightness" is the mean of R, G, and B component of brightness. The patterns of series C have the same brightness distribution as the patterns of series A. The two panels at the bottom indicate the positions of the horizontal sections.

Figure 5. Power spectrums of horizontal brightness distributions of each visual stimulus patterns in Fig.3 (ordinate: arbitrary scale). Power spectrums of the patterns of series C are the same as those of series A.



Tables.

Table 1. Responses to series A stimulus patterns

[Display index]

Pattern	Average Display index	Multiple comparison			
		S-F15	S-S	S-F45	S-A
S-O	13.2	0.01	0.01	0.002	0.002
S-F15	8.8		n.s.	0.005	0.002
S-S	5.8			0.05	0.005
S-F45	3.9				0.005
S-A	0.3				

[Movement index]

Pattern	Average Movement index (pixels)	Multiple comparison			
		S-F15	S-S	S-F45	S-A
S-O	5.719x10 <sup>5</sup>	0.02	0.05	0.002	0.002
S-F15	4.271x10 <sup>5</sup>		n.s.	0.05	0.002
S-S	3.817x10 <sup>5</sup>			0.01	0.005
S-F45	2.671x10 <sup>5</sup>				0.02
S-A	1.786x10 <sup>5</sup>				

[Area index]

Pattern	Average Area index (pixels)	Multiple comparison			
		S-F15	S-S	S-F45	S-A
S-O	9.162x10 <sup>5</sup>	0.02	0.002	0.002	0.002
S-F15	7.915x10 <sup>5</sup>		0.05	0.02	0.02
S-S	6.528x10 <sup>5</sup>			n.s.	n.s.
S-F45	6.520x10 <sup>5</sup>				n.s.
S-A	6.530x10 <sup>5</sup>				

n.s.: no significance, Wilcoxon's signed rank test, N=12  
 Numbers in the multiple comparison part indicate that the pattern in the left side column is significantly more effective than the pattern in the upper column at this significance level. The same applies to the following tables.

Table 2. Responses to series B stimulus patterns

[Display index]

Pattern	Average Display index	Multiple comparison			
		C-SG	C-S	C-G	C-A
S-O	13.3	0.02	0.01	0.005	0.005
C-SG	9.4		n.s.	0.01	0.002
C-S	8.9			0.01	0.002
C-G	4.0				n.s.
C-A	2.8				

[Movement index]

Pattern	Average Movement index (pixels)	Multiple comparison			
		C-SG	C-S	C-G	C-A
S-O	5.426x10 <sup>5</sup>	0.02	0.005	0.002	0.002
C-SG	4.379x10 <sup>5</sup>		n.s.	0.01	0.01
C-S	3.878x10 <sup>5</sup>			0.05	0.02
C-G	2.561x10 <sup>5</sup>				n.s.
C-A	2.368x10 <sup>5</sup>				

[Area index]

Pattern	Average Area index (pixels)	Multiple comparison			
		C-SG	C-S	C-G	C-A
S-O	8.759x10 <sup>5</sup>	0.005	0.005	0.002	0.002
C-SG	7.269x10 <sup>5</sup>		n.s.	0.005	0.01
C-S	6.876x10 <sup>5</sup>			n.s.	n.s.
C-G	6.359x10 <sup>5</sup>				n.s.
C-A	6.104x10 <sup>5</sup>				



Table 3. Responses to series C stimulus patterns

[Display index]

Pattern	Average Display index	Multiple comparison		
		SBW-F15	SBW-F45	SBW-A
SBW-O	12.1	0.002	0.002	0.002
SBW-F15	4.9		0.02	0.002
SBW-F45	2.1			0.05
SBW-A	0.7			

[Movement index]

Pattern	Average Movement index (pixels)	Multiple comparison		
		SBW-F15	SBW-F45	SBW-A
SBW-O	6.064x10 <sup>5</sup>	0.005	0.002	0.002
SBW-F15	2.846x10 <sup>5</sup>		n.s.	n.s.
SBW-F45	2.175x10 <sup>5</sup>			n.s.
SBW-A	2.125x10 <sup>5</sup>			

[Area index]

Pattern	Average Area index (pixels)	Multiple comparison		
		SBW-F15	SBW-F45	SBW-A
SBW-O	8.762x10 <sup>5</sup>	0.005	0.002	0.002
SBW-F15	6.582x10 <sup>5</sup>		n.s.	n.s.
SBW-F45	6.133x10 <sup>5</sup>			n.s.
SBW-A	6.108x10 <sup>5</sup>			

## Series A

## Series B

S-O



S-O



S-F15



C-SG



S-F45



C-G



S-A



C-A



S-S



C-S





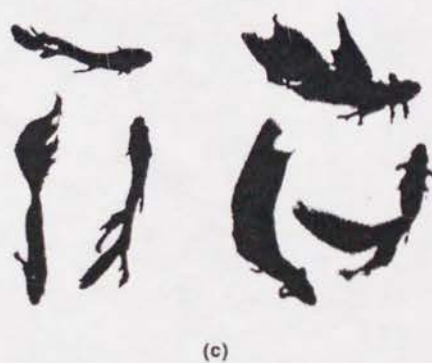
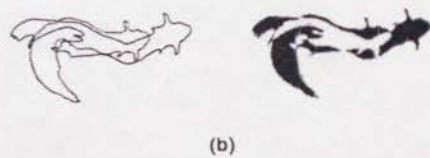
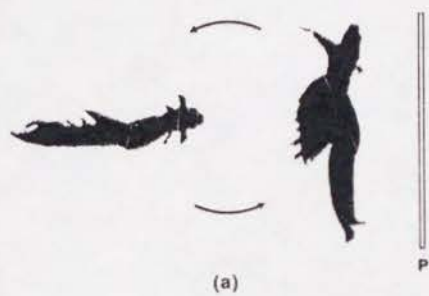


Fig. 2

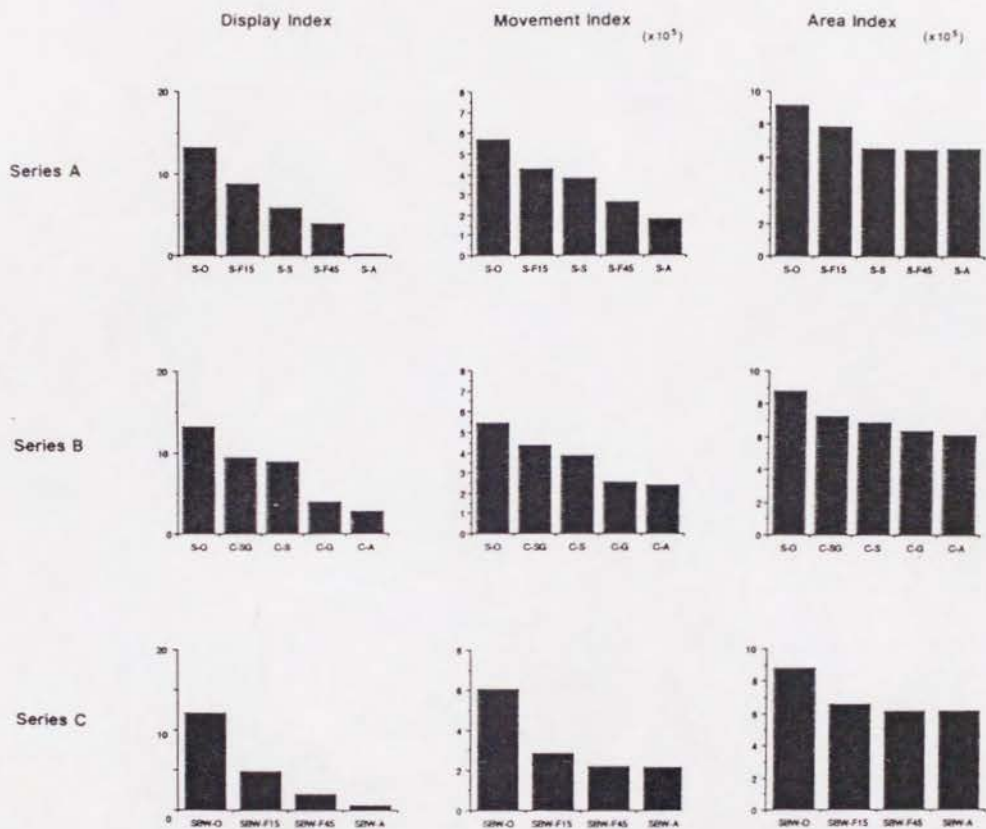


Fig. 3



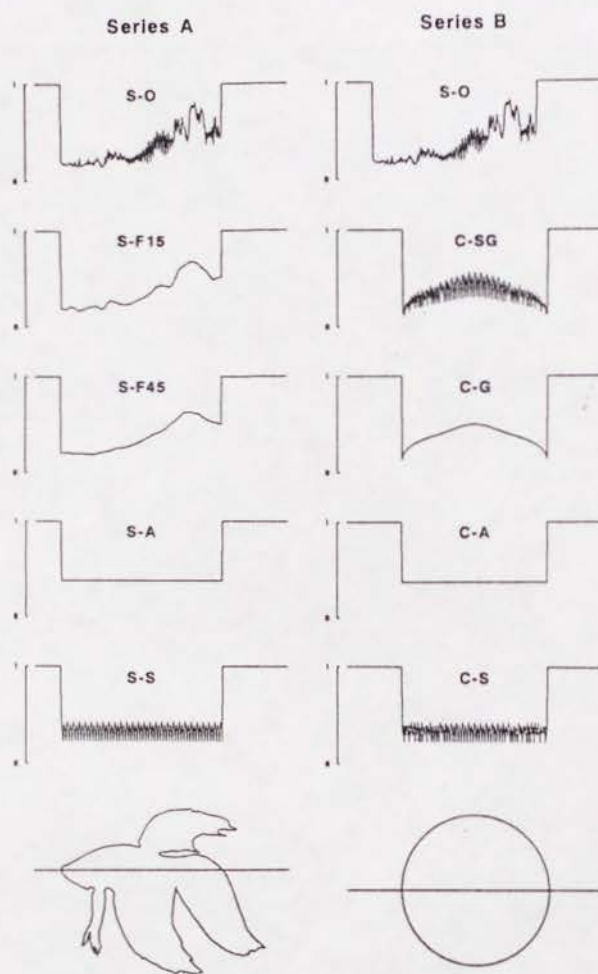


Fig. 4

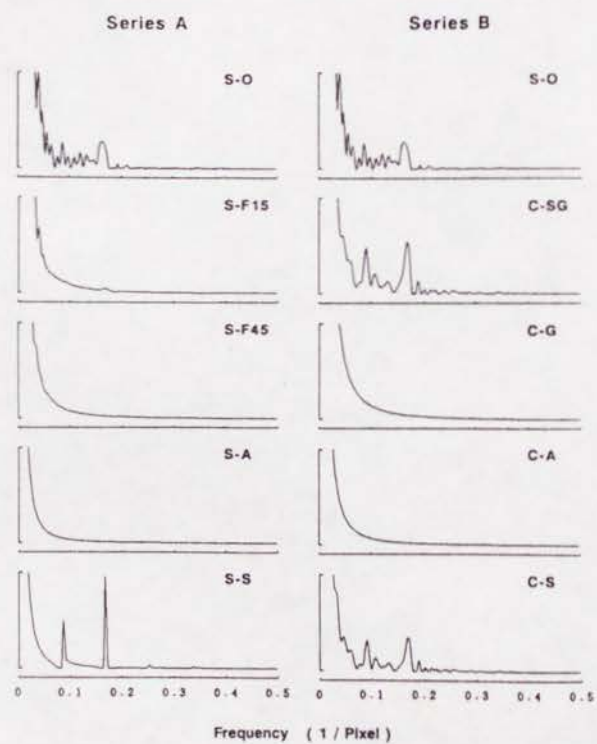


Fig. 5